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14. ABSTRACT

The 2009 Pennington Scientific Symposium entitled "Circadian Biology and Sleep": Missing Links in Obesity and Metabolism? Was held at the Lod Cook Conference Center on the Louisiana State University Campus on 4/27-28, 2009. Over 25 invited speakers and their guests together with ~15 to 20 faculty members from the Pennington Biomedical Research Center attended the sessions which integrated current findings in the fields of circadian biology, metabolism, nutrition, and sleep. In addition to the keynote address by Dr. Joseph Takahashi, a total of 14 full talks and 2 short talks were presented. A round-table discussion was conducted at the conclusion of each day's meeting. Each speaker was given the option to submit his/her talk in manuscript format to the journal *Obesity Reviews*; over 75% of the speakers have indicated that they will publish in this special supplement issue.

15. SUBJECT TERMS
Circadian biology; metabolism; molecular biology; obesity; sleep

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Introduction:
We now live in a mechanized world that is increasingly divorced from the physical environment. With artificial lighting, we no longer experience a 10-12 hour period of true darkness at night. Our sleep cycles and activity periods have become divorced from the earth's rotational rhythm. Night shift workers might be eating lunch at 2 AM and sleeping at 2 PM. With modern air travel, we now experience "jet lag" as our bodies are forced abruptly to acclimate to new time zones and altered light:dark cycles. There will be increasing circadian stresses placed on the U.S. civilian population's lifestyle as globalization forces factories, retail commerce, and computer-based jobs towards a "24/7" operating schedule. Nevertheless, the comprehensive integration of the fields of circadian biology, sleep, metabolism, nutrition, and human physiology remains a challenge for investigators, clinicians, and administrators in both the civilian and military settings.

Body:
To address the important questions concerning the role of circadian mechanisms in human disease and to foster inter-disciplinary communication and collaboration, a symposium entitled *"Circadian Biology and Sleep: Missing Links in Obesity and Metabolism?"* was held at the Lod Cook Conference Center on the campus of the Louisiana State University (Baton Rouge, LA) between April 26-29, 2009. The co-Chairs for the meeting were Andrew Young, PhD, from the U.S. Army Research Institute of Environmental Medicine , Molly Bray, PhD, from the Baylor College of Medicine, and Jeffrey M. Gimble, MD PhD, from the Pennington Biomedical Research Center. Each of these investigators has expertise in the field of human performance, circadian biology, adipose tissue, and/or obesity research.

The following distinguished panel of scientists from the fields of circadian biology, sleep, human performance, and obesity research attended this meeting and presented their talks entitled as listed below.

Keynote Address: Joseph Takahashi, PhD (Northwestern University):
Uncovering the Molecular Basis of Circadian Biology

Full Talks:

Joseph Bass, MD, PhD
Northwestern University
Obesity and Metabolism in the Clock Mutant Mice: Lessons for Human Metabolism

Molly Bray, PhD
Baylor College of Medicine
Genetic Variation in Circadian Genes: Relation to Obesity and Adipocyte Biology

Andrew Butler, PhD
The Scripps Research Institute
The role of melanocortin neuronal pathways in circadian biology: a new homeostatic output involving melanocortin-3 receptors?

Nicolas Cermakian, PhD
McGill University
Regulation of central and peripheral circadian rhythms in mice and humans

James Gangwisch, PhD
Columbia University
The Epidemiological Relationships Between Sleep Patterns and Metabolic Disorders

Jeff Gimble, MD, PhD
Pennington Biomedical Research Center
Food Entrainment and the Circadian Regulatory Apparatus in Peripheral Tissues

Carla Green, PhD
University of Virginia
Nocturnin: A Novel Circadian and Metabolic Regulator

Jonathan Johnston, PhD
University of Surrey
Adipocyte rhythmicity in vitro

Tracy Rupp, PhD
Walter Reed Army Institute of Research
Long-term effects of nightly sleep duration on cognitive performance, alertness, metabolism and obesity

Jared Rutter, PhD
University of Utah
Nutrient sensing and signaling: Roles in energy balance and elsewhere

Albert Stunkard, MD
University of Pennsylvania
A Biobehavioral Model of the Night Eating Syndrome

Gregory Sutton, PhD
Pennington Biomedical Research Center
Maternal Imprinting and Metabolism: A Circadian Relationship?

Alexandros Vgontzas, MD
Penn State College of Medicine
Meaningful subtyping of Obesity based on Sleep Disturbances

Martin Young, PhD
Baylor College of Medicine
The Circadian Biology of Cardiac Tissues: A Metabolic Model for Understanding Human Metabolic Syndrome

Short Talks:

Karyn Esser, PhD
University of Kentucky,
Circadian Biology of Skeletal Muscle

Alok Gupta, MD
Pennington Biomedical Research Center,
Circadian Variation of Blood Pressure in Obese and Diabetic Subjects

Each of the invited speakers was encouraged to bring a junior colleague or collaborator to the meeting to participate in the proceedings and a total of 11 additional individuals from academia and industry attended the symposium. Additionally, the Pennington Biomedical Research Center faculty were invited to participate in the conference. Between 10-20 of the full time faculty members at the Center joined the meeting in addition to post-doctoral fellows, research associates, and staff members. The meeting sponsors had invited Dr. Michael Twery, a sleep specialist and program officer at the National Heart, Lung, and Blood Institute in Bethesda, MD, to attend. Although he had originally accepted and was on the program, his commitments at the NIH dealing with the stimulus package forced him to change his plans. Likewise, Dr. Andrew Young's other commitments prevented him from attending. In his place, Thomas Balkin, PhD, Chief, Department of Behavioral Biology, Walter Reed Army Institute for Research, attended the meeting and chaired one of the symposium sessions.

Arne Astrup, the editor in chief of the prestigious journal ***Obesity Reviews***, agreed to publish papers from the symposium. Each of the presenting scientists was invited to contribute an article based on their presentation. These articles will appear in a special issue of the journal which has an ISI impact factor of 7.821, higher than any other publication in the specialized field of obesity research. These publications, along with an overview of the meeting prepared by the organizers, will serve to disseminate the findings and conclusions of the conference to the broader scientific community. At least 75% of the speakers have agreed to submit articles based on their presentations. The meeting co-chairs and Dr. Balkin will prepare a summary statement on the meeting objectives and outcomes for this special supplement to ***Obesity Reviews***.

Key Research Accomplishments:

1. State of the art review of:
    a. Circadian Biology at the Basic and Translational Levels
    b. Nutrition and Obesity
    c. Sleep Medicine
2. Cross Training of Investigators From Different Disciplines
3. Fostering of Inter-Disciplinary Collaborations
4. Development of a Special Supplement Issue of Obesity Reviews with Manuscripts from the Symposium

Reportable Outcomes:

The following reportable outcomes have or will result from this funding:
Abstract Booklet (attached)
Obesity Review Special Supplement with manuscripts from >75% of the symposium speakers and summary statement from symposium co-chairs (to be completed)

Conclusions:

The symposium sponsored by the US Army Medical and Materiel Command offered an outstanding educational forum that allowed exchange of ideas between scientists from different disciplines coming from laboratories in the US, Canada, and the United Kingdom. The event highlighted the existing knowledge and future directions for circadian biology in the context of human disease and performance relevant to both the military and civilian population. In addition, the event initiated dialogues with the potential to lead to collaborative efforts between US Army and academic laboratories, both national and international. Much of the information related at the symposium will be made available to the scientific community through its publication in a Special Supplement Issue of *Obesity Reviews*. This opportunity was only possible through the generous support of the US Army.

Pennington Scientific Symposium

Circadian Biology and Sleep: Missing Links in Obesity and Metabolism?

April 27-28, 2009

Symposium Chairs

Molly Bray, PhD
Associate Professor of Pediatrics
Baylor College of Medicine
Children's Nutrition Research Center

Jeffrey Gimble, MD, PhD
Professor, Stem Cell Biology
Pennington Biomedical Research Center
Louisiana State University System

Andrew Young, PhD
Chief, Military Nutrition Division
U.S. Army Resarch Institute of Environmental Medicine

This symposium was coordinated by the
Division of Education

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Phillip J. Brantley, PhD, Director
Anne Schulte, Assistant Director

Monday, April 27

SESSION CHAIR:	**Molly Bray, PhD - Molecular Mechanisms in Circadian Biology and Metabolism**
8:00-8:30	**Claude Bouchard, PhD** *Pennington Biomedical Research Center: Mission, Structure, Programs and Financing*
8:30-9:30	**Joseph Takahashi, PhD** Northwestern University *Keynote Address: Uncovering the Molecular Basis of Circadian Biology*
9:30-10:15	**Joseph Bass, MD, PhD** Northwestern University Feinberg School of Medicine *Obesity and Metabolism in the Clock Mutant Mice: Lessons for Human Metabolism*
10:15-10:30	**BREAK**
10:30-11:15	**Carla Green, PhD** University of Virginia *Nocturnin: A Novel Circadian and Metabolic Regulator*
11:15-12:00	**Jared Rutter, PhD** University of Utah School of Medicine *Nutrient sensing and signaling: Roles in energy balance and elsewhere*
12:00 – 1:00	**LUNCH**
SESSION CHAIR:	**Jeff Gimble, MD, PhD - Circadian Biology of Sleep**
1:00-1:45	**Albert Stunkard, MD** University of Pennsylvania *A Biobehavioral Model of the Night Eating Syndrome*
1:45-2:30	**Nicolas Cermakian, PhD** McGill University *Regulation of central and peripheral circadian rhythms in mice and humans*
2:30-2:45	**BREAK**
2:45-3:30	**Jonathan Johnston, PhD** University of Surrey *Adipocyte rhythmicity in vitro*
3:30-4:15	**James Gangwisch, PhD** Columbia University *The Epidemiological Relationships Between Sleep Patterns and Metabolic Disorders*
4:15-5:00	**Round Table Discussion**
5:00	**Hospitality Suite**
6:30	**Tour and Dinner for Out of Town Attendees - PBRC**
9:00	**Hospitality Suite**

Tuesday, April 28

SESSION CHAIR:	**Tom Balkin, PhD – Central & Peripheral Metabolic Tissues and Circadian Mechanisms**
8:30-9:15	**Andrew Butler, PhD** The Scripps Research Institute *The role of melanocortin neuronal pathways in circadian biology: a new homeostatic output involving melanocortin-3 receptors?*
9:15-10:00	**Molly Bray, PhD** Baylor College of Medicine *Genetic Variation in Circadian Genes: Relation to Obesity and Adipocyte Biology*
10:00 – 10:15	**BREAK**
10:15-11:00	**Gregory Sutton, PhD** Pennington Biomedical Research Center. LSU System *Maternal Imprinting and Metabolism: A Circadian Relationship?*
11:00-11:45	**Jeff Gimble, MD, PhD** Pennington Biomedical Research Center, LSU System *Food Entrainment and the Circadian Regulatory Apparatus in Peripheral Tissues*
11:45-12:45	**Lunch**
SESSION CHAIR:	**Michael Twery, PhD - Integrating Sleep, Metabolism, and Circadian Pathways**
12:45-1:30	**Martin Young, PhD** Baylor College of Medicine *The Circadian Biology of Cardiac Tissues: A Metabolic Model for Understanding Human Metabolic Syndrome*
1:30-2:15	**Tracy Rupp, PhD** Walter Reed Army Institute of Research *Long-term effects of nightly sleep duration on cognitive performance, alertness, metabolism and obesity*
2:15-2:30	**BREAK**
2:30-3:15	**Alexandros Vgontzas, MD** Penn State College of Medicine *Meaningful subtyping of Obesity based on Sleep Disturbances*
3:15-3:45	**Michael Twery, PhD** National Heart, Lung, and Blood Institute *The NIH Perspective*
3:45 - 5:00	**Consensus Discussion** *Address pre-symposium "critical questions in the field" and any additional questions raised during the meeting. Develop consensus for meeting report document.*
5:00	**Hospitality Suite**
7:00	**Dinner - Lod Cook Alumni Center**
9:00	**Hospitality Suite**

Uncovering the Molecular Basis of Circadian Biology

Joseph S. Takahashi, PhD
Howard Hughes Medical Institute, UT Southwestern Medical Center, Dallas, TX

Circadian rhythms represent an evolutionarily conserved adaptation to the environment that can be traced back to the earliest life forms. In animals circadian behavior can be analyzed as an integrated system - beginning with genes leading ultimately to behavioral outputs. In the last decade, the molecular mechanism of circadian clocks has been uncovered by the use of phenotype-driven (forward) genetic analysis in a number of model systems. Circadian oscillations are generated by a set of genes forming a transcriptional autoregulatory feedback loop. In mammals, these include: *Clock, Bmal1, Per1, Per2, Cry1, Cry2* and *Casein kinase 1 epsilon*. Another dozen candidate genes have been identified and play additional roles in the circadian gene network such as the feedback loop involving *Rev-erba*. Despite this remarkable progress, it is clear that a significant number of genes that strongly influence and regulate circadian rhythms in mammals remain to be discovered and identified. As part of a large-scale N-ethyl-N-nitrosourea (ENU) mutagenesis screen using a wide range of nervous system and behavioral phenotypes, we have identified new circadian mutants in mice. These include new alleles of known circadian genes as well as novel circadian loci such *Fbxl3*, an orphan member of the F-box protein family, which we have found to interact selectively with the CRY proteins to target them for degradation through the proteasome pathway.

The discovery of 'clock genes' also led to the realization that the capacity for circadian gene expression is widespread throughout the body. Using circadian gene reporter methods, one can demonstrate that most peripheral organs and tissues can express circadian oscillations in isolation, yet still receive and may require input from the dominant circadian pacemaker in the suprachiasmatic nucleus (SCN) *in vivo*. We have used tissue-specific, conditional gene expression methods to analyze the relative contributions of central and peripheral circadian oscillators to circadian organization. The cellular autonomy of circadian clocks has raised a number of questions concerning synchronization and coherence of rhythms at the cellular level as well as circadian organization at the systems level. I will discuss recent work that addresses these issues and that examines a number of levels of complexity within the circadian system.

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Obesity and Metabolism in the Clock Mutant Mice: Lessons for Human Metabolism

Joseph T. Bass, MD, PhD
Northwestern University, Evanston, IL

The circadian system orchestrates the temporal organization of many aspects of physiology and metabolism in synchrony with the 24 hr rotation of the Earth. Like the metabolic system, the circadian system is a complex feedback network that involves interactions between the brain and peripheral tissues. While the core clock resides in the master pacemaker neurons of the suprachaismatic (SCN) nucleus, the entire clock network is expressed within both extra-SCN regions of the brain and in peripheral tissues.

Emerging evidence suggests that circadian regulation is intimately linked to metabolic homeostasis and that circadian dysregulation contributes to obesity and diabetes. At the molecular level, we have recently found that rhythmic NAD^+ biosynthesis completes a novel metabolic feedback loop through which the positive limb of the clock leads to activation of the NAD^+-dependent deacetylase Sirt1 which in turn inhibits Per2 transcription. These results pinpoint NAD^+ biosynthesis and Sirt1 activity as a critical node in the molecular integration of circadian and metabolic networks. Studies of the crosstalk between circadian and metabolic systems have opened new understanding of obesity and diabetes mellitus.

Nocturnin: A Novel Circadian and Metabolic Regulator

Carla Green, PhD
University of Virginia, Charlottesville, VA

Nocturnin is a deadenylase that controls mRNA expression in a circadian manner by degrading the poly-A tails of target mRNAs, leading to mRNA turnover or translational silencing (1, 2). Previously we reported that a mouse lacking Nocturnin was resistant to diet-induced obesity and heptatic steatosis (3). The lean phenotype was not due to increased activity, decreased food intake or a higher metabolic rate. Transcript analysis in liver showed alterations in genes associated with lipid uptake and utilization.

In an effort to discern the mechanism behind this lean phenotype, we examined digestive tract function. Our initial studies indicated that the Nocturnin KO mice a faster transit time for lipid, but not water, in a gut motility assay. We therefore hypothesized that Nocturnin has a role in the absorption of dietary lipid in bowel. In order to investigate the mechanism further, we exposed WT and KO mice to various dietary challenges. When subjected to either a ketogenic diet or food restriction, our KO mice lost more weight than their WT counterparts. When on a standard mouse chow, the KO mouse exhibited lower circulating beta-hydroxybutyrate – a finding consistent with altered lipid availability in the KO. Moreover, this latter discrepancy in the KO is not due to hepatocyte malfunction as hepatocyte analysis showed normal rates of both lipid uptake and beta-oxidation.

We then examined aspects of digestive tract function directly by giving the mice a lipid bolus and performing histological examinations. The lipid droplets formed along the jejunum of the small intestine were significantly larger in the Nocturni KO mice than in the WT mice. Through a subsequent series of *in vivo* and *in vitro* studies, we demonstrated that the KO mice are deficient in their ability to take up lipids. These animals have significantly disrupted lipid trafficking in the enterocytes, resulting in decreased absorption via apoB-containing non-HDL lipoproteins. We propose that Nocturnin has a role in the absorption of dietary lipid in bowel, presumably by altering genes necessary for metabolism or digestion through circadian post-transcriptional modifications of targeted transcripts.

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Nutrient Sensing and Signaling: Roles in Energy Balance and Elsewhere

Jared Rutter, PhD
University of Utah, Salt Lake City, UT

The PAS domain is a sequence motif originally identified in and named for the transcription factors Period, the Aryl hydrocarbon receptor (AHR), and SIM. The aryl hydrocarbon receptor is present in the cytoplasm of normal cells. Xenobiotics, such as dioxin, bind to the PAS domain of AHR, and cause its translocation to the nucleus and activation of specific target genes. The PAS domain has subsequently been found in a large number of proteins from all phyla. Many of these proteins are the sensor proteins of bacterial two-component systems, which are responsible for responding to many metabolic and environmental stimuli, such as oxygen tension, redox, and light. In general, the PAS domain is thought to be a regulator of the activity of other domains within the protein. My work over the last decade and the work of my lab over the last five years has been focused on the PAS domain and a few of the proteins that contain and are regulated by PAS domains.

NPAS2 and Clock are basic helix-loop-helix PAS transcription factors that function in the circadian clock (Antoch et al., 1997; King et al., 1997; Reick et al., 2001). Since the identification of NPAS2 by colleagues in the McKnight lab (Zhou et al., 1997), we hypothesized, by analogy with the aryl hydrocarbon receptor, that it would be a ligand-activated transcription factor. Toward identifying putative ligands, we expressed and purified the PAS domain-containing portion of the protein. Interestingly, the protein when expressed in bacteria co-purified with a chromophore with an absorbance maximum of 420nm. We identified the chromophore as heme and mapped the interaction to the PAS domain of NPAS2. We were able to show that carbon monoxide regulated DNA binding activity of NPAS2 and Clock through interaction with the bound heme moiety (Dioum et al., 2002). In the intervening seven years, others have repeated and extended on these studies (Ishida et al., 2008; Koudo et al., 2005; Mukaiyama et al., 2006; Uchida et al., 2005). It is quite clear that the in vitro observations are reproducible. What is much less clear is the physiological relevance of these observations in the control of circadian rhythms, although recent mutagenesis studies have implicated heme binding in transcriptional activity in cultured cells (Ishida et al., 2008).

PAS kinase is an evolutionarily conserved nutrient-responsive kinase. PASK consists of a PAS regulatory domain followed by a canonical ser/thr kinase domain (Rutter et al., 2001). As with many other PAS domain proteins, the activity of PASK is controlled by metabolic status. Studies in yeast and mammalian cells have demonstrated that PASK activity is nutrient-regulated and controls the decision whether to store or utilize available nutrients (da Silva Xavier et al., 2004; Grose et al., 2007) (Smith and Rutter, 2007) (Rutter et al., 2002a). In order to understand the functions of PASK in mammals, we are studying *PASK*$^{-/-}$ mice. *PASK*$^{-/-}$ mice developed normally, but had altered metabolic parameters. The most dramatic observation was that *PASK*$^{-/-}$ mice were resistant to high-fat diet induced obesity, hepatic steatosis and insulin resistance (Hao et al., 2007). We have focused on the role of PASK in regulating hepatic lipid metabolism and have identified a transcriptional pathway by which it might be mediating its effects. These findings suggest an important physiological role of PASK in regulating metabolism and controlling energy balance in mammals (Hao and Rutter, 2008). They also raise the possibility that PASK inhibition would be an effective therapeutic strategy for metabolic disease.

Previous colleagues and I proposed in 2002 that circadian rhythms might be, ancestrally and fundamentally, a metabolic cycle that has been reinforced by an elegant regulatory system of transcriptional, phosphorylation and protein degradation control (Rutter et al., 2002b). The connections between circadian rhythms and metabolism are certainly abundant and powerful (see (Green et al., 2008) for a recent review). As we have begun to understand some of the metabolic bases of obesity and diabetes, the issues of energetic efficiency, rhythmic activity of mitochondria and metabolic enzymes, rhythmic availability of energetic substrate and others have become very interesting areas where circadian rhythms might be interacting with metabolic regulation in highly disease-relevant scenarios.

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A Biobehavioral Model of the Night Eating Syndrome

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The Night Eating Syndrome is an eating disorder consisting of evening hyperphagia (at least 25% of daily caloric intake after supper) and/or two awakenings at night with ingestions (1). It represents a delayed circadian rhythm of meal times of about 1.5 hours, with a similar delay in neuroendocrine functions (2). Prevalence of NES has varied considerably in the past depending upon the criteria which had changed. At the present time, the two criteria noted above have been widely accepted and data on prevalence is based on these criteria. In community samples it varies from 1.1% (3) to 1.6% (4) with higher prevalence reported among obese patients in weight loss samples (5) and even higher prevalence among preoperative bariatric surgery patients (5). One of the highest prevalences was found in psychiatric clinics with increased risk for patients with substance abuse disorders (6). The only population-based study of the prevalence of NES, based on the Swedish Twin Registry, found a prevalence of night eating among 4.6% of men and 3.4% of women (7). This, and other studies, found a strong relationship between body weight and the prevalence of night eating.

One large prospective study showed that nighttime overeaters gained significantly weight (6.2 kg) than did non-night eaters (1.7 kg; p=0.03) (8). NES is believed to result from an interaction of genetic vulnerability (7) and stress which has been consistently reported as a precipitating factor. The result of this interaction is an elevation in density of serotonin transporters in the midbrain, which transport serotonin from the synapse to the cell body, leaving a deficit of serotonin for neurotransmission (9). This deficit leads to the disruption of normal circadian rhythm of meal times and neuroendocrine functions. This model is supposed by four studies of selective serotonin reuptake inhibitors (SSRIs), which inhibit the reuptake of serotonin and increase its presence at the synapse (10-13). This treatment has been effective in controlling night eating. It also resulted in weight loss, the only situation in which SSRIs have produced weight loss and indicates that this action is via control of NES.

Despite the ease of diagnosis of NES and the presence of effective treatment, the disorder is not widely recognized. A survey of 103 night eaters indicated that 90% of them said that their doctors hadn't heard of NES and 40% said that their doctors had told them that their complaints were of no consequence (14).

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Regulation of Central and Peripheral Circadian Rhythms in Mice and Humans

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Many circadian rhythms are controlled by the central clock of the suprachiasmatic nucleus (SCN) of the hypothalamus[1]. However, many other brain regions and most peripheral tissues have also been shown to function as autonomous clocks[2]. The role of these peripheral clocks and their regulation by environmental and SCN-derived signals is still unclear. We address these questions in both mice and humans, and more specifically, we study circadian rhythms in cells of the immune system.

Our studies in mice focus on circadian rhythms in lymph nodes (LNs). T cell proliferation after antigen presentation in LNs is the first step in the adaptive immune response[3]. Serum levels of cytokines and circulating immune cells were previously shown to vary with a 24-hour circadian rhythm[4,5]. Also, the persistence of rhythmic immune parameters under constant light conditions, under which no central clock-driven rhythms are present, suggested that there is a clock in the immune system[5]. We hypothesized that a circadian clock in LNs controls T cell function. Our aims were to identify clock gene expression in mouse LNs, investigate T cell proliferation rhythms, and examine LN rhythms in mice with a deficient clock. Adult male C57BL/6 mice were housed under a light:dark cycle, placed in constant darkness (DD), and sacrificed on the second day in DD every 4 h over 24 h. LNs were sampled and used to: i) Extract RNA and quantify clock gene expression by real-time PCR; or ii) Measure T cell proliferation following T cell receptor stimulation with an anti-CD3 antibody in vitro. Similar experiments were performed with *Clock* mutant mice and wild-type littermates. We show that clock genes are rhythmically expressed in lymph nodes and that the clock controls T cell proliferation. T cells undergo more divisions in response to stimulation when sampled from mice in the early night compared to other times of day, and this variation is lost in cells collected from *Clock* mutant mice. We are currently investigating intracellular signaling pathways downstream of the T cell receptor to assess the mechanisms behind this circadian rhythm in the proliferative response. This is the first evidence of a clock in LNs, and of control of the immune response by the molecular clockwork. Our results have uncovered a novel mode of regulation of T-cell proliferation. These studies will shed light on the links between circadian rhythms, immune response and cancer. They may also provide cues for more efficient vaccination strategies.

The rationale of our studies in humans is to use clock gene expression in peripheral blood mononuclear cells (PBMCs) as a model of peripheral oscillator, and to compare the regulation of central and peripheral rhythms under different environmental conditions. In a first study, we used an uninterrupted 72-h sampling period to compare the expression of peripheral circadian oscillators in PBMCs in the presence of a habitual sleep/wake cycle (LD) and under constant routine (CR) conditions[6]. Six healthy men (n=4) and women (n=2, follicular phase) aged 18-30 years (mean age ± SEM: 23.7 ± 1.6) maintained a regular 8-hour sleep episode for two weeks prior to the study. Repeated whole blood samples were drawn from an indwelling catheter during a 72-hour period including 40 h of LD where subjects were exposed to 118±8 lux of polychromatic light during daytime wake periods and slept in darkness, and a 32-h CR of sustained wakefulness and limited activity under dim light. Real-time PCR was used to assess expression of *PER1*, *PER2* and *BMAL1* in PBMCs isolated from whole blood samples every ~120 min, relative to *CDK4*. Plasma melatonin sampled every ~60 min and continuously (~1/min) sampled core body temperature (CBT) were used as markers of the central circadian pacemaker. The CBT minimum and the midpoint of melatonin concentration were well aligned to the sleep/wake cycle. *PER1* and *PER2* expression in PBMCs demonstrated significant circadian rhythmicity that peaked early after wake time and was comparable under LD and CR conditions. *BMAL1* expression was more variable, and peaked in the middle of the wake period under LD conditions and during the habitual sleep period under CR conditions. These results demonstrate that the pattern of clock gene expression in PBMCs can be evaluated over extended periods, and the pattern of *PER1* and *PER2* expression are in stable relationships with markers of the central circadian pacemaker.

Judicious light and darkness exposure throughout the day can promote the appropriate alignment of the endogenous hormonal rhythms to night shift work[7]. However, the synchronization of human peripheral

circadian oscillators to shifted sleep-wake schedules is currently unknown. Thus, building on the results of our previous experiment, we evaluated clock gene expression in PBMCs with respect to the simultaneous resetting of the plasma cortisol and melatonin rhythms throughout simulated night shift work[8]. Five healthy candidates (4 male, 1 female in follicular phase) aged (mean ±SD) 24.9 ±4.8 years maintained stable sleep and meal schedules before the study start. Upon admission to the laboratory, sleep/wake schedules were delayed by 10 h to simulate nighttime "work". The light intervention included exposure to polychromatic white light of (mean±SEM) 6,036±326 lux during 8-h night shifts and dim light exposure after each night shift with the use of sunglasses (5% visual light transmission). Phase of clock gene expression in PBMCs, and plasma melatonin and cortisol concentration were estimated from 24-h blood sampling periods performed before and after nine simulated night shifts. The expression of clock genes *PER1*, *PER2* and *BMAL1* was determined as above. Following nine days on the night schedule, melatonin and cortisol rhythms regained their conventional alignment with the shifted sleep/wake schedule. Changes in the pattern of clock gene expression were apparent when sampled as early as after three days on the shifted sleep/wake schedule. Following nine days on the night schedule, *PER1* and particularly *PER2* expression in PBMCs displayed significant circadian rhythmicity and were a conventional alignment with the shifted sleep/wake schedule, with peak expression occurring during the wake period. This is the first demonstration of the entrainment of a human peripheral circadian oscillator to an atypical sleep-wake schedule. This line of investigation has important implications for understanding the medical disorders affecting night shift workers.

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Adipocyte Rhythmicity In Vitro

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White adipose tissue (WAT) plays a key role in multiple aspects of physiology. In addition to storing energy in the form of triglycerides, WAT is now known to act as an active endocrine tissue via the synthesis and secretion of adipokines (1). Many aspects of endocrine physiology and metabolism are under the control of endogenous circadian clocks and there is now growing evidence for the circadian regulation of adipose function (2-5).

Analysis of transgenic mouse models provides a compelling physiological link between molecular components of the circadian clock and metabolism. Even though the resulting phenotype appears partially dependent on genetic background, mutation of clock-related genes consistently results in a metabolic dysregulation, including dyslipidaemia (6-9). Similar genetic links to metabolism have been found in humans, with clock gene polymorphisms being associated with obesity, metabolic syndrome and type 2 diabetes mellitus (10-12).

Within WAT, rhythmic gene expression is widespread and an estimated 20% of the murine WAT transcriptome, including adipokine mRNA, exhibits diurnal fluctuation (2,13-15). Furthermore, circadian regulation of endocrine activity is likely to be an important output of WAT, as studies of both rodents and humans have revealed 24-hour variation of adipokine concentration in the blood (16-19). Despite this emerging understanding of adipose rhythmicity, a number of key issues remain unanswered. Of these, my group is particularly focussed on *in vitro* and human physiological models.

To date, most studies of adipose rhythmicity have analysed *ex vivo* WAT derived from rodents maintained on a light-dark cycle. WAT is heterogeneous and contains multiple cell types in addition to the primary endocrine cells, the adipocytes. *In vivo*, WAT is subject to multiple rhythmic input signals, which are both humoral and neuronal in nature (20-23). Finally, there is a possibility of environmental 'masking' of physiological rhythms when individuals are housed in fluctuating environmental conditions. The cellular basis of reported adipose rhythms is therefore unclear and in need of further investigation. A major focus of current work is to characterise rhythmicity in isolated pre-adipocyte and adipocyte cells. Many of these studies are being performed in collaboration with Prof Gary Frost and other colleagues at Imperial College, London.

There have to date only been limited attempts to translate rodent data on WAT rhythms to human studies. I am currently leading a study, in collaboration with Prof Debra Skene and Dr Denise Robertson, to evaluate endocrine rhythms and WAT gene expression in physiologically characterised human subjects.

I will present preliminary data from the above studies and provide an overview of the links between clocks, sleep, metabolism and nutrition within the Sleep and Chronobiology group at the University of Surrey.

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The Epidemiological Relationship Between Sleep Patterns and Metabolic Disorders

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In this country we are experiencing parallel trends of increasing body mass index, increasing prevalence of hypertension and diabetes, and decreasing average sleep duration. There is reason to believe that these trends are linked by a mismatch between our modern lifestyles and our metabolic regulatory systems that evolved to be functional when the lifestyles of our hunter-gatherer ancestors were quite different from today. The thrifty genotype is hypothesized to have evolved through natural selection to be expressed during seasons of high food availability to induce high blood insulin, insulin resistance, and glucose-intolerance to facilitate increased fat deposition to adaptively prepare for seasons of food scarcity. The central biological clock evolved to synchronize activity, consumption, and rest to the circadian and circannual cycles using hormones and the autonomic nervous system. To generate and organize autonomic rhythms, the SCN requires repeated metabolic cues from light exposure, sleep, activity, and nutrient intake. Reliable environmental cues of the impending seasons for our nomadic ancestors would have been the length of the daily photoperiod that changed in a precise and predictable fashion throughout the year and the intake of seasonally available nutrients. Longer photoperiods in summer would have corresponded with shorter sleep durations and higher availability of food and simple sugars. Short sleep durations could therefore help trigger the expression of the thrifty genotype to increase caloric intake and fat deposition. Today we can have year round short sleep durations with electric lights and year round availability of highly palatable foods and simple sugars, so we can have year round fat accumulation. The seasonal expression of the thrifty genotype is therefore consistent with metabolic changes resulting from chronically short sleep durations leading to diseases associated with the metabolic syndrome such as obesity, diabetes, and hypertension.

Experimental studies have found sleep deprivation to increase appetite by decreasing leptin and increasing ghrelin levels. Inadequate sleep and poor-quality sleep are associated with obesity in children, adolescents, and adults studied in case-control, cross-sectional, and longitudinal studies[1-3]. These relationships are stronger in children, adolescents and young adults. Associations in the elderly have been found with more accurate measures of sleep duration and adiposity.

Sleep deprivation in experimental studies has been shown to decrease glucose tolerance and compromise insulin sensitivity. It has been suggested that habitually short sleep durations could lead to insulin resistance by increasing sympathetic nervous system activity, raising evening cortisol levels, and decreasing cerebral glucose utilization. The increased burden on the pancreas from insulin resistance can, over time, compromise β-cell function and lead to type 2 diabetes. Longitudinal studies have found U-shaped relationships between sleep duration and diabetes incidence, with both short and long sleep durations being associated with diabetes incidence.[4] It is likely that long sleep duration occurs in parallel to, and as a consequence of, diabetes and other conditions associated with chronic inflammation. Proinflammatory cytokines contribute toward sleepiness and fatigue, have deleterious effects on both glucose homeostasis and β-cell function, and are elevated in obesity and in conditions in which the primary pathogenic mechanism is insulin resistance.

Sleep restriction has been shown to acutely increase blood pressure and sympathetic nervous system activity in both normotensive and hypertensive subjects. Blood pressure dips by an average of 10 to 20% during sleep, so shorter sleep durations increase hemodynamic load by raising average 24-hour blood pressure and heart rate. Shorter sleep durations also result in longer exposures to waking physical and psychosocial stressors. Increased exposure to stress has been shown to promote salt appetite and suppress renal salt-fluid excretion. Long-term exposure to increased total 24-hour hemodynamic load associated with short sleep durations can lead to structural adaptations that gradually reset the entire cardiovascular system to operate at an elevated pressure equilibrium. Short sleep duration has been found to be associated with the prevalence of hypertension in cross-sectional studies and the incidence of hypertension in longitudinal studies.[5]
A u-shaped relationship between sleep duration and mortality has consistently been found in epidemiologic studies, with both short and long sleep durations being associated with increased mortality. The association between long sleep and mortality has been viewed as justification by some investigators to recommend sleep

restriction to decrease mortality risk despite the fact that no published laboratory or epidemiologic studies have demonstrated a possible mechanism identifying long sleep as a cause of either morbidity or mortality. In analyses stratified by age, the u-shaped relationship between sleep duration and mortality was found only in elderly subjects and not in middle-aged subjects.[6] Results from these analyses indicate that the U-shaped relationship is heavily influenced by deaths in elderly subjects and by the measurement of sleep durations closely before death. Subjects' sleep durations reported closely before death are unlikely to be indicative of their sleep durations over the course of their lifetimes, when they develop chronic diseases that eventually contribute toward death. It is likely that the sleep durations of the elderly subjects who died were affected by the presence of medical conditions that eventually contributed toward their deaths. The majority of deaths in elderly subjects are attributable to major cardiovascular diseases, cancer, and diabetes. The inflammatory process has been shown to play key roles in both the pathogenesis and pathophysiology of cancer and of metabolic disorders such as cardiovascular disease and type 2 diabetes. The elderly subjects' immune responses to these conditions could have resulted in a predominance of pro-inflammatory cytokines that functioned to lengthen sleep, implying that long sleep duration is unlikely to contribute toward mortality but, rather, is a consequence of conditions associated with chronic inflammation and age-related sleep changes.

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The Role of Melanocortin Neuronal Pathways in Circadian Biology: A New Homeostatic Output Involved Melanocortin-3 Receptors?

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Energy homeostasis involves maintaining a balance of appetite with energy expenditure. The neural melanocortin system has a critical role in this process, and is involved in regulating both satiety and energy expenditure [1,2] . In the brain, the melanocortin system is composed of neurons that secrete the endogenous melanocortin receptor ligands, and/or that express melanocortin receptors. The melanocortin receptor family is comprised of 5 g-protein coupled receptors (Mc1-5r), with Mc3r and Mc4r expressed in areas of the central nervous system involved in energy homeostasis. The melanocyte stimulating hormones (□-,□- and □-MSH) exhibit agonist activity, and are products of the post-translation processing of proopiomelanocortin (Pomc). Agouti-related peptide (AgRP) was originally described as antagonist for the Mc3r and Mc4r, although this neuropeptide also acts as an inverse agonist. Neurons that express either *Pomc* or *AgRP* mRNA are located in the arcuate nucleus of the hypothalamus, and send projections to other neural centers involved in regulating complex behavior and the neuroendocrine and autonomic outputs that govern metabolism and energy expenditure. The hypothalamic melanocortin system responds rapidly to food intake in rats restricted to a once-daily feeding regime, suggesting acute regulation by peripheral inputs of mean intake [3]. The regulation of satiety and energy expenditure by melanocortin ligands predominantly involves Mc4r. Activation of Mc4r reduces food intake and increases energy expenditure [1,2]. However, while having an important function in maintaining normal adiposity [4], the exact role of the Mc3r in energy balance regulation has remained enigmatic.

My laboratory recently reported evidence suggesting that Mc3r are involved in entrainment to food intake [5]. The rationale for examining the role of melanocortins in food entrainment was based on the early studies demonstrating that hypothalamic lesions significantly impair entrainment of rats to a single meal [6]. Using *Mc3r-/-* mice backcrossed >10 generations onto the C57BL/6J (B6) strain [3], we observed significant impairment of entrainment to restricted feeding (RF: food available 1300-1700h in mice housed in a 12 h light:dark setting, with lights on 0600-1800h). Several readouts of activity and arousal were used to monitor entrainment. First, we observed using wheel cages that the development of food anticipatory activity (FAA) was significantly impaired in *Mc3r-/-* mice. Second, we examined spontaneous locomotor activity (SLA)using beam breaks in the x- and z-axes using the Comprehensive Laboratory Animal Monitoring System (CLAMS). We observed a peak in SLA in control B6 mice subject to RF centered on food presentation which was markedly reduced in *Mc3r-/-* mice. Using electroencephalography (EEG) and electromyography (EMG), we then observed that a period of wakefulness in control mice coinciding with FAA and anticipating meal presentation was absent in *Mc3r-/-* mice.

FAA is dependent on the activity of genes encoding proteins involved in maintaining the rhythm of molecular clocks. Mice lacking either the Clock paralog neuronal PAS domain protein 2 (*Npas2*) or Period2 (*Per2*) exhibit impaired FAA when subject to restricted feeding protocols [7,8]. We observed abnormal rhythms of expression of critical clock genes in the forebrain of *Mc3r-/-* mice. Importantly, we were able to demonstrate similar abnormal rhythms of clock activity and impaired adaptation to RF in wild type B6 mice administered intracerebroventricularly with AgRP, a Mc3r antagonist/inverse agonist.

Collectively, these data suggest that one function of the Mc3r is to regulate inputs into systems involved in the development of behaviors that are entrained to food availability. We have also determined that Mc3r have a critical role in maintaining a normal rhythm of activity in the liver clock during RF. Using the same technology applied to the Mc4r [9], we are now developing a condition mutant, the LoxTB-Mc3r mouse, to further define the role of neural Mc3r in regulating entrainment.

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Genetic Variation in Circadian Genes: Relation to Obesity and Adipocyte Biology

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Obesity is one of the most profound public health problems today, and though much has been learned regarding the regulation of body weight and the development of adiposity, the prevalence of obesity continues to rise. Simplistic explanations based on nutritional over-consumption and/or poor diet or lack of physical activity are inadequate to account for this dramatic and literal growth in our population, demonstrating the urgent need for new insight into mechanisms that may lead to obesity and altered energy homeostasis. Although it is well established that genetic variation plays a substantial role in the determination of body size and adiposity, the contribution of environmental alterations that have taken place in the last 20-30 years cannot be ignored. In addition to an increase in the abundance of low-quality, highly palatable, energy dense food, combined with a decrease in opportunities for exercise and physical activity, the average time spent sleeping is also decreasing. Recently, a number of studies have supported a link between the altered sleep/wake/eating patterns associated with our "24 hour" lifestyle and obesity.[1] According to the 2008 Annual Report from the National Sleep Foundation, obese individuals on average spent less time in bed (6.8 vs. 7.2 hours) and were more likely to sleep less than 6 hours per night (19% vs. 12%) compared to non-obese individuals. At present, the mechanisms by which altered daily rhythms are translated into increased adiposity are unknown.

Biological rhythms, such as sleep/wake cycles, are an integral component of virtually all aspects of life. These rhythms are controlled in large part by circadian clocks, intrinsically maintained molecular mechanisms that serve to condition the organism to changes in its environment. Though many studies have investigated the function of the central clock in the regulation of circadian physiology, our understanding of the role of peripheral clocks in whole body energy metabolism is just beginning to emerge. Recent studies have provided evidence that both central and peripheral circadian clocks likely regulate many physiologic functions, including insulin sensitivity, endocrine regulation, energy homeostasis, satiety signaling, and cellular proliferation.[2-5] The phenotypic outcomes of circadian clock disruption are varied, with sleep deprivation and shift work associated with insulin resistance, increased hunger, alterations in adipocytokines, and increased risk for obesity, cardiovascular disease, and type 2 diabetes.[6-8]

Disruption of the circadian clock can result from alterations in the DNA sequence of clock component or clock-regulated genes and/or loss of synchrony between natural patterns of light/dark, sleeping, and eating. In animals, disruption of clock-component genes has been associated with altered feeding patterns, hormonal abnormalities, alterations in blood lipids and adipokines, alterations in lean and fat mass, defective glucose homeostasis, and reduced life span.[4,5,9-11] Limited studies to date have investigated variation in the clock-component or clock-regulated genes for association to body fat and obesity in humans. Multiple variants in CLOCK have been reported to be associated with the development of the metabolic syndrome, type 2 diabetes and cardiovascular disease.[12-14] DNA sequence variants in CLOCK, CK1E, GSK3B, PER2, and PER3 have been associated with sleep and mood disorders, bipolar disease, seasonal affective disorder, advanced sleep phase syndrome, depression, and morning-evening preference.[15-19] While obesity has been associated with a number of these conditions, limited studies specifically investigating the role of variation within circadian clock genes in human obesity have been reported to date. The combination of genetic alterations in the circadian clock and behavioral dyssynchrony with the 24-h environment may have profound effects on overall health. This presentation will provide an overview of the current knowledge of the role of clock-associated genetic mutation/variation in the development of obesity and related co-morbidities.

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Maternal Imprinting and Metabolism: A Circadian Relationship?

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The mechanisms linking intrauterine growth retardation (IUGR) with adulthood obesity and diabetes are unknown. These studies investigated energy metabolism in 8 wk and 5 month old male and female mice subjected to protein malnutrition in utero. We investigated the metabolic phenotype of IUGR offspring prior to the onset of obesity. We also investigated weather B6 mice subjected to prenatal protein deficiency also develop signs of Type II Diabetes. Undernourished pups from pregnant C57BL/6J dams fed a protein deficient diet (6 % protein, UO) were cross-fostered to lactating dams fed normal breeder chow. UO exhibited lower birth weight (≈60% of normal weight), but displayed rapid catch-up growth. 8 wk old chow-fed UO mice exhibit improved glucose clearance relative to CO. However, UO exhibited marked abnormalities in circadian rhythms of wheel running, feeding behavior and metabolic activity. Specifically, food intake, energy expenditure and the respiratory exchange ratio (RER) were increased in UO during the lights-on period. Expression of genes involved in hepatic lipid and glucose metabolism revealed that expression of Rev-erbα in liver was dramatically reduced in UO at 2 mo of age. Rev-erbα repressed genes involved in circadian regulation (Bmal1, Per2) and inflammation (plasminogen-activator inhibitor-1) were increased in UO mice. The same profile of gene expression was observed in male UO at 5 mo of age. UO mice exhibit a metabolic disorder involving abnormal circadian patterns of feeding behavior, increased lipogenesis and inflammation prior to obesity. Loss of Rev-erbα expression and function may be a key factor in metabolic dysregulation associated with IUGR.

Food Entrainment and the Circadian Regulatory Apparatus in Peripheral Tissues

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Classical studies of human obesity and adipogenesis have focused on the transcriptional regulators C/EBPα and PPARγ2 and their downstream target genes relating to glucose and lipid metabolism. There is a growing awareness that circadian regulatory mechanisms play a contributory role in adipose tissue function. Studies in murine models have demonstrated the oscillatory expression of mRNAs encoding core circadian regulatory proteins (CCRP) in adipose depots [Ando et al., 2005; Bray and Young, 2007; Ptitysn AA, 2006; Zvonic et al., 2006]. The adipose expression profile of the CCRP mRNAs can be significantly phase shifted by temporal restriction of food access [Goh BC and Hill MR, 2007]. In some obesity models, the CCRP oscillatory expression profile in adipose tissue is attenuated [Ando et al., 2005]. The same CCRP mRNAs were detected in subcutaneous adipose tissue of human subjects (n = 151). Analysis of the female Caucasian subjects (n = 116) found that expression of DBP, E4BP4, Rev-erbα, and PGC1β all showed a trend ($p < 0.1$) or significant ($p < 0.05$) correlation with BMI in lean or young overweight/obese subjects; however, in older overweight/obese subjects, no correlation between CCRP mRNAs and BMI was detected. Consistent with these findings, human adipose derived stem cells (ASCs), capable of adipogenic, chondrogenic, and osteogenic differentiation, displayed a synchronized expression of CCRP mRNAs following exposure to dexamethasone or serum shock [Wu et al., 2007]. Similar observations were obtained in studies using bone marrow derived mesenchymal stem cells (BMSCs) from humans or mice [Wu et al., 2008]. Together, these studies indicate that human adipose tissue expresses the CCRP mRNAs in an oscillatory manner similar to that documented in murine models.

As observed in other peripheral, metabolically active tissues, at least 20% of the expressed mRNAs in subcutaneous adipose tissue exhibit an oscillatory expression profile [Ptitysn AA, 2006; Zvonic et al., 2006]. A comparison of oscillatory mRNA in the transcriptome of four tissues (brown adipose, calvarial bone, liver, subcutaneous white adpose) revealed a shared set of ~180 transcripts common to each [Zvonic et al., 2007]. These included all members of the CCRP displayed on the microarray as well as mRNAs encoding critical rate limiting enzymes in glucose, iron, and lipid metabolism. An additional transcript in this shared subgroup displayed the intriguing name of "delta sleep inducing peptide immunoreactant (DSIPI)". Also known as "glucocorticoid induced leucine zipper (GILZ)", DSIPI was a protein first identified by antibodies directed against the nine amino acid delta sleep inducing peptide. The delta sleep inducing peptide was first discovered in the serum of sleeping mammals and was found to cause sleep when infused at high concentrations into otherwise awake animals. Pennington research Abba Kastin MD performed some of the pioneering work on delta sleep inducing peptide and demonstrated that its serum levels exhibited a robust circadian oscillation over a quarter century ago [Fischman et al., 1984; Graf and Kastin, 1984]; however, the protein source of the peptide remains unknown. Studies by Shi and his colleagues at the Medical College of Georgia have documented a regulatory role of DSIPI (GILZ) in the reciprocal regulation of adipogenesis and osteogenesis in BMSCs [Shi et al., 2003; Zhang et al., 2008]. In light of the growing interest in the relation between obesity risk and sleep, both DSIP and GILZ offer promising targets for future investigations in both pre-clinical and clinical models.

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The Circadian Biology of Cardiac Tissues: A Metabolic Model for Understanding Human Metabolic Syndrome

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Diurnal variations have been firmly established in both cardiovascular physiology (e.g. heart rate, cardiac output) and pathophysiology (e.g. arrhythmias). As with myocardial contractile function, myocardial metabolism exhibits a marked diurnal variation. These phenomena have been attributed primarily to diurnal variations in extracardiac influences, including various neurohumoral factors, such as sympathetic and autonomic stimulation. Virtually every mammalian cell possesses an intrinsic circadian clock, a transcriptionally-based molecular mechanism capable of regulating multiple cellular functions. Circadian clocks allow individual cells to perceive the time of day. In doing so, this cell autonomous molecular mechanism confers the selective advantage of anticipation, enabling both rapid and appropriate responses to environmental stimuli upon their onset.

CLOCK mutant mice, which exhibit altered diurnal variations at multiple levels (including feeding-fasting cycles, neurohumoral factors), have been reported to develop multiple features of the Cardiometabolic Syndrome, suggesting that circadian clocks play critical roles in energy homeostasis. We have recently generated a cardiomyocyte-specific circadian clock mutant (CCM) mouse, as a means to identify the influences that this molecular mechanism has on myocardial physiology and pathophysiology. This model has exposed the cardiomyocyte circadian clock as a critical modulator in the responsiveness of the heart to fatty acids, at transcriptional, metabolic, and contractile function levels. We recently reported that the rodent heart exhibits increased susceptibility to fatty acid-induced contractile dysfunction during the less active/sleep (light) phase, a phenomenon that may be due to increased channeling of fatty acids into non-oxidative 'lipotoxic' pathways at this time. In contrast, exposure of the rodent heart to fatty acids during the more active/awake (dark) phase is associated with promotion of fatty acid oxidation, via transcriptional mechanisms. Through the use of CCM mice, studies within our laboratory suggest that both diurnal variations in fatty acid channeling, and in the transcriptional responsiveness of the heart to fatty acids, are mediated, at least in part, by the circadian clock within the cardiomyocyte. For example, the cardiomyocyte circadian clock directly regulates myocardial triglyceride metabolism, as evidenced by attenuated triglyceride syntheses for CCM hearts both *in vivo* (fasting-induced) and *ex vivo* (working mouse heart perfusions), through regulation of multiple triglyceride metabolism genes (e.g. *dgat2, adpn*).

Armed with the aforementioned knowledge, we have initiated studies designed to investigate whether restricting dietary lipid to distinct times of the day influences multiple parameters of the Cardiometabolic Syndrome. These studies suggest that restricting dietary lipid to the initiation of the more active/awake (dark) phase provides significant benefit at the levels of body weight gain/adiposity, glucose tolerance, skeletal muscle insulin sensitivity, and myocardial contractile function.

Taken together, our studies show that the circadian clock within the cardiomyocyte allows the heart to anticipate environmental stimuli (such as fatty acids), and that synchronization between the myocardium and its environment is enhanced by distinct meal feeding regimes. Impairment of the cardiomyocyte circadian clock (as observed during diabetes mellitus, ischemic heart disease) results in a loss of synchronization between the heart and its environment, potentially predisposing the myocardium to metabolic maladaptation and subsequent contractile dysfunction. Similarly, dyssynchronization of dietary lipid intake and circadian clock-mediated peripheral tissue fatty acid oxidation promotes Cardiometabolic Syndrome development.

Long-term Effects of Nightly Sleep Duration on Cognitive Performance, Alertness, Metabolism, and Obesity

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American adults report sleeping an average of 6.8 hours on weeknights[1]—considerably less than the 8 h of sleep thought to be necessary to restore and sustain optimal daytime alertness. Whereas total and partial sleep loss each produce similar effects on objectively measured alertness and performance, the time course for recovery from chronic partial sleep loss is relatively extended.[2] Evidence also suggests that chronic sleep restriction leads to physiological changes that may be linked to obesity and diabetes.[3,4,5,6] According to a review by Knutson and colleagues,[7] the relationship between sleep loss, weight gain, and diabetes risk involves multiple pathways; 1) alterations in glucose, 2) upregulation of appetite, and 3) decreased energy expenditure.

Here we present evidence that the extent to which sleep restriction impairs objectively measured alertness and performance, and the rate at which these impairments are subsequently reversed by recovery sleep, varies as a function of the amount of nightly sleep obtained prior to the sleep restriction period.[8] We then discuss implications of the present findings for understanding the potential effects of sleep extension and partial sleep loss on metabolism and obesity; including that prior sleep extension might mediate (and potentially buffer) the multiple pathways connecting sleep loss to weight gain and diabetes risk, specifically with regards to alterations in glucose and the upregulation of appetite.

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Obesity and Sleep Disturbances

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Obesity, excessive daytime sleepiness (EDS), and self-reported short sleep duration appear to be on the rise, while there is evidence that obesity and these sleep disorders are strongly connected. In our presentation, we will review data that challenge the common belief that the sleep apnea and sleep loss, frequently associated with obesity, are the primary determinants of obesity-related objective daytime sleepiness and subjective fatigue (tiredness without increased sleep propensity). Specifically, obesity is associated with objective and subjective EDS regardless of the presence of sleep apnea. The association between obesity and EDS was confirmed in recent studies of large random samples of the general population or clinical samples, which showed that the primary determinants of subjective EDS were depression, metabolic disturbances, i.e., obesity/diabetes and insulin resistance, and lack of physical activity, and, secondarily, sleep apnea or sleep loss. Paradoxically, within the obese, with or without sleep apnea, those who slept objectively better at night are sleepier (objectively) during the day than those who slept worse. The distinguishing factor between those that slept better vs. those that slept worse appears to be level of emotional stress. Furthermore, many studies reported that obesity is associated with self-reported short sleep duration; however, it appears that short sleep duration is a marker of emotional stress rather than a reflection of true sleep loss. Based on these data, we propose that obesity-related deeper sleep and objective EDS are primarily related to metabolic disturbances, whereas obesity-related poorer sleep and subjective fatigue appear to be the result of psychological distress.

Furthermore, based on data from studies in normal controls and patients with sleep disorders, it appears that the interaction of the hypothalamic-pituitary-adrenal (HPA) axis and pro-inflammatory cytokines determines the level of sleep/arousal within the 24-hour cycle, i.e., "eucortisolemia" or "hypocortisolemia" plus hypercytokinemia is associated with high sleep efficiency and objective sleepiness, whereas "hypercortisolemia" plus hypercytokinemia is associated with low sleep efficiency and fatigue.

In conclusion, we propose that the above-reviewed data provide the basis for a meaningful phenotypic and pathophysiologic subtyping of obesity. One subtype is associated with emotional distress, poor sleep, fatigue, HPA axis "hyperactivity," and hypercytokinemia while the other is associated with nondistress, better sleep but more sleepiness, HPA axis "normo or hypoactivity," and hypercytokinemia. This proposed subtyping may lead to novel, preventive and therapeutic strategies for obesity and its associated sleep disturbances.

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Discussion Questions:

1. Although the impact of clock gene disruption on metabolism and related health problems has been highlighted by a number of studies, a recurring question is whether the observed defects are due to clock dysfunction (e.g. via deregulated clock-controlled genes) or to clock-independent roles of these so-called clock genes. The same question applies to all other health issues occurring upon clock gene dysfunction.

2. Does sleep extension a) protect against the effects of subsequent sleep loss on metabolism and/or b) help reverse metabolic effects associated with prior sleep debt?

3. Are there age-related changes in the effects of chronic sleep on metabolism and obesity?

4. What specific countermeasures (pharmacological or behavioral) might protect against the metabolic effects of chronic insufficient sleep?

5. How might laboratory versus field studies best capture the long-term consequences of chronic insufficient sleep on metabolism?

6. Are the metabolic phenotypes observed in the various clock-deficient mice the result of loss of the clock per se, or due to other pleiotropic effects of the clock genes (or a combination of the two)?

7. What consideration are people giving to background strain when conducting metabolic studies in circadian transgenics?

8. What is the evidence that the night eating syndrome involves a disturbance of the glucocorticoid system, the melatonergic system and the serotonergic system?

9. What do you think is the reason for the lack of knowledge on the part of physicians of this easily diagnosed and readily treated disorder?

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